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Molecular Evaluation of Viral load and Genotype of HCV in Acute and Chronic HCV Patients in Kirkuk City

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Abstract

The study aimed at detection of HCV load and genotypes in acute and chronic HCV patients. A cross sectional study was carried out in Kirkuk city from 15th of March 2017 to 15th of November 2017. The number of hepatitis patient understudy were 62 hepatitis C (27 acute and 35 chronic) whose ages were between 20-75 years old. These patients admitted to Hepatology and Gastroenterology centers of Kirkuk. The control group who were matched to the patients studied, included 30 individuals who admitted to blood bank for blood donation.

The study showed that no statistical differences between acute and chronic HCV concerning their viral load and the highest viral load mean was found in acute HCV patients (1162.6 v.s 1234.3) IU/ml. In the current study, the high rates of chronic and acute HCV patients were infected by genotype 4 of HCV (68.69% and 63.63% respectively) and the lowest rate were genotype 1a. The study showed that there was a highly significant difference between viral loads of acute and chronic HCV patients as regarding genotype 1a (1298.7 v.s. 1155.4 IU/ml), (P: 0.001), the study showed a significant difference between viral loads of acute and chronic HCV patients concerning genotype 4 and a significant relation of viral loads of acute HCV infection with genotype 1a and genotype 4. It was concluded that the was no difference HCV load in acute and chronic infection and genotype 4 as the most frequent HCV genotype in Kirkuk

Keywords: HCV, genotype, viral load, Kirkuk.

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التقييم الجزيئي للحِمل الفيروسي والنمط الوراثي لفيروس التهاب الكبد نوع C في مرضى التهاب الكبد الفيروسي ذوي الاصابات الحادة والمزمنة في مدينة كركوك

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الملخص

تهدف الدراسة الى الكشف الكمي والجيني لفايروس التهاب الكبد نوع C في المرضى ذوي الإصابات الحادة والمزمنة. أجريت الدراسة في مدينة كركوك للفترة من 15 شباط 2017 ولغاية 15 تشرين الثاني 2017. وشملت 62 مصاباً بالتهاب الكبد نوع C (27 إصابة حادة و35 مزمنة) تراوحت أعمارهم (20–75) سنة والذين راجعوا مركز الكبد والجهاز الهضمي في كركوك. كانت مجموعة السيطرة عبارة عن 30 فردا الذين كانوا يراجعون مصرف الدم الرئيسي للتبرع بالدم. أظهرت الدراسة ان لا وجود لفرق احصائي بين المرضى ذوي الإصابات الحادة والمزمنة بما يخص كمية الفايروس في الدم (1162.6 و1234.3) وحدة دولية/مل. كما أظهرت النتائج ان اعلى نسبة من المرضى ذوي الإصابات المزمنة والحادة كانوا مصابين بالفيروس ضمن الصنف الجيني الرابع (68.69% و 63.63% على التوالي) وان اقل نسبة من الإصابات كانت ضمن الصنف الجيني a1. أظهرت الدراسة ان هنالك فرقا معنويا بين معدلات كميات الفايروس في مرضى الإصابات الحادة والمزمنة بما يخص الصنف الجيني a (1155.4 و1298.7) وحدة دولية /مل (P:0.001) وان هنالك علاقة معنوية بين معدلات كميات الفايروس في مرضى الإصابات الحادة والمزمنة بما يخص الصنف الجيني الرابع وان هنالك علاقة قوية بين الصنفين الجينيين في المرضى. يستنتج من الدراسة ان لا وجود لفرق معنوي بين الإصابات الحادة والمزمنة بما يخص الفحص الكمي وان الصنف الجيني الرابع هو الصنف الأكثر شوعا في مرضى التهاب الكبد نوع C في مدينة كركوك

الكلمات الدالة: التهاب الكبد الفيروسي نوع C; الصنف الجيني; الحِمل الفيروسي; كركوك.
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1. Introduction:

Viral hepatitis is a general term describing liver infection caused by viruses actively replicating in the liver [1]. Like hepatitis caused by other agents, such as alcohol and drug abuse or metabolic disorders, a typical feature of the disease is jaundice [2]. Acute hepatitis is a transient episode of inflammatory liver disease. During this phase of up to six months, the disease may be clinically unapparent, or accompanied by clinical features ranging from mild symptoms such as jaundice, nausea and malaise to severe symptoms such as liver failure or death [3]. Chronic hepatitis, in which markers of the disease can be found for a period longer than six months, has the same range of clinical outcomes seen in acute hepatitis [4]. The disease of hepatitis C is with a significant global impact, it infects more than 170 million people worldwide. Infections with HCV are pandemic, World Health Organization (WHO) estimates a worldwide prevalence of 3% [5]. HCV is classified into six major genotypes (designated 1-6), many subtypes (designated a, b, c, etc.), and about 100 different strains (numbered 1,2,3, etc.) based on the genomic sequence heterogeneity [6]. Genotypes 1-3 have a worldwide distribution. Types 1a and 1b are the most common, accounting for about 60% of global infections. They predominate in Northern Europe and North America, and in Southern and Eastern Europe and Japan, respectively. Type 2 is less frequently represented than Type 1. Type 3 is endemic in south-east Asia and is variably distributed in different countries [7]. Genotype 4 is principally found in the Middle East, Egypt, and central Africa. The determination of the infecting genotype is important for the prediction of response to antiviral treatment: genotype 1 and 4 are generally associated with a poor response to interferon alone, whereas genotypes 2 and 3 are associated with more favorable responses[8].

2. Material and Methods:

A cross sectional study was carried out in Kirkuk city from 15th of March 2017 to 15th of November 2017. The number of hepatitis patient understudy were 62 hepatitis C (27 acute and 35 chronic) whose ages were between 20-75 years old. Patients who newly diagnosed and their infection was up to six months were considered acute infections and those who exceed six months were considered chronic infection [1]. These patients admitted to Hepatology and Gastroenterology centers of Kirkuk and they were previously diagnosed positive by ELISA. The control group who were matched to the patients studied, included 30 individuals who

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were apparently haven't any diseases who admitted to blood bank for blood donation and they previously diagnosed negative by ELISA.

2.1 Methods:

Five ml of blood was collected by vein puncture, the obtained plasma were aspirated and transferred to Eppendorf tubes and stored in deep freeze at -20°C for late molecular test of HCV (viral load and genotype) using Sacace™ Biotechnology /HCV Real-TM Quant. Extraction of HCV-RNA was done by using of zymo research Viral RNA Kit which provides for rapid isolation of high-quality viral RNA from a wide range of biological sources. It can be used to successfully isolate viral RNA from cell-free body fluids as well as cellular suspensions at concentrations ≤1x105 cells/ml. The ZR Viral RNA Kit™ employs a single buffer system that facilitates viral particle lysis and allows for RNA adsorption onto the matrix of the Zymo-Spin™ Column. The RNA is washed then eluted with DNase/RNase-Free water. The HCV Real-TM Quant is a Real-Time test for the quantitative detection of Hepatitis C virus in human plasma by using specific primers designed by the HCV Real-TM Quant (Sacace™ Biotechnology) and included in the kit for HCV qualitative detection. (Genotype plus Real-TM, Sacace Biotechnology, Italy). HCV genotype Plus Real-TM is based on 2 major processes:

1. Reverse transcription of the RNA.
2. Real time PCR:

•PCR-mix-1-FRT HCV 1b/3 with primers and probes for subtypes 1b, 3.
•PCR-mix-1-FRT HCV 1a/2 with primers and probes for subtypes 1a, 2.
•PCR-mix-1-FRT HCV 4/IC with primers and probes for subtypes 4 and Internal Control.
•PCR-mix-1-FRT HCV 5a/6 with primers and probes for subtypes 5a, 6.

2.2 Statistical Analysis:

Computerized statistically analysis was performed using IBM SPSS V23.0.0 statistic program.

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3. Results:

Table 1 showed that 81.48 % of acute HCV patient who previously diagnosed by ELISA were positive by PCR and 82.86 % of chronic HCV patients were positive by PCR while no one of the control group were positive by PCR with highly significant relation.

Table 1: Comparison between ELISA and PCR in testing of HCV.

Anti HCV ELISA positive	Real-time PCR assay				Total samples	
	Positive		Negative			
	No.	%	No.	%	No.	%
Acute HCV (n: 27)	22	81.48	5	18.52	27	100
Chronic HCV (n:35)	29	82.86	6	17.14	35	100
Control group (n:30)	0	0	30	100	30	100
P. value = 0.00001						

Table 2 showed no statistical differences between acute and chronic HCV concerning their viral load and the highest viral load mean was found in acute HCV patients (1162.6 v.s 1234.3) IU/ml .

Table 2: Relation of HCV load with acute and chronic HCV infection.

Parameters	N	Mean (IU/ml)	SD	*P. value*
Acute HCV	22	1162.6	197.4	0.20
Chronic HCV	29	1234.3	196.22	

In the current study, HCV patients divided in to two part, one infected with genotype 1a and the other was infected with genotype 4 and there was no coinfection between them. The study showed that the high rates of chronic and acute HCV patients were infected by genotype 4 of HCV (68.69% and 63.63% respectively) and as compared with patients with genotype 1a, Fig.1.

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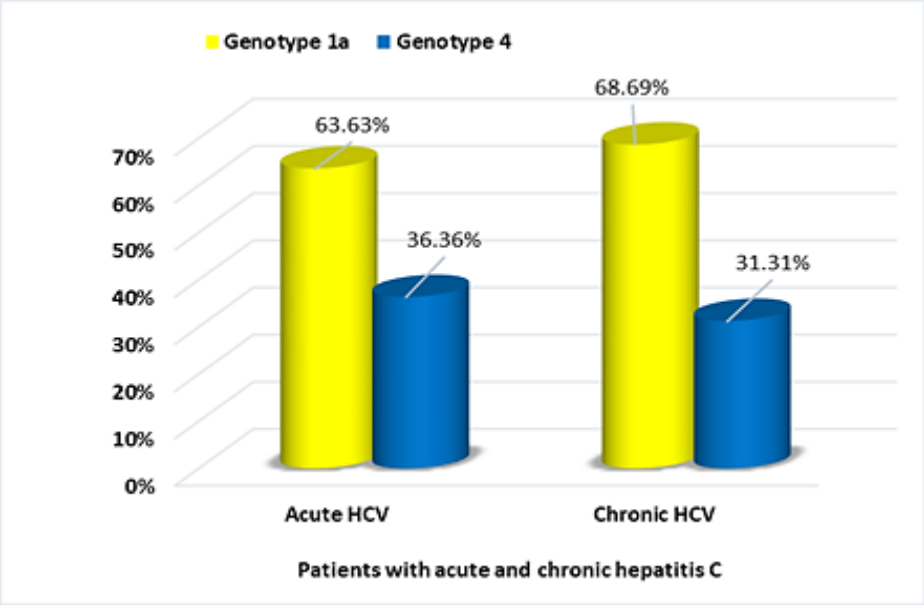

Fig.1: Distribution of patients with acute and chronic hepatitis C according to HCV genotypes.

The study showed that there was a highly significant difference between viral loads of acute and chronic HCV patients as regards genotype 1a (1298.7 v.s. 1155.4 IU/ml), (P: 0.001), a significant difference between viral loads of acute and chronic HCV patients concerning genotype 4 and a significant relation of viral loads of acute HCV infection with genotype 1a and genotype 4, Table 3.

Table 3: Relation of viral load with genotype in patients with acute and chronic hepatitis C.

HCV genotype	HCV Viral load (IU/ml)				*P. value*
	Patients with acute hepatitis C (n:20)		Patients with chronic hepatitis C (n:29)		
	No.	Mean ± SD	No.	Mean ± SD	
Genotype 4	14	1298.7±134.7	20	1155.4±195.5	0.001
Genotype 1a	8	1121.6±245.8	9	1198.8±206.5	0.047
P. value	0.032		0.57		

4. Discussion:

The EASL guidelines suggest that individuals who qualify for HCV testing should sequentially be tested for HCV antibodies and, if positive, this should be followed by a confirmatory HCV RNA test. The indications for testing with an HCV nucleic acid test include a positive HCV serology (to confirm current active HCV infection), patients who are

immunocompromised (including those on chronic hemodialysis) even if HCV serology is negative, or individuals exposed to HCV within the last 6 months who may not have developed detectable HCV antibodies. In addition, HCV RNA testing should be performed in all patients with HCV undergoing treatment with antiviral therapy and at regular intervals during and after treatment [1]. Barreiro et al [2] demonstrated that HCV-RNA viral load was 6000.02 IU/ml in chronic hepatitis patients. Probably as many as 70%-90% of infected people fail to clear the virus during the acute phase of the disease and become chronic carrier [3]. The study agreed with Osoba et al [5] in Kingdom of Saudi Arabia (KSA) who determine the prevalence of HCV genotype in various population group in KSA and genotype 4 was recorded as the most common genotype ranging from 40%-74% among the various group of patients studied genotype of HCV. In particular, examination of sequence diversity can help understand the different patterns of serological reactivity, response to treatment [4]. There is substantial evidence that HCV possesses different pathogenic potentials, since different responses to interferon treatment depending on the HCV type/subtype have been reported [6].

The pattern of our genotypes is similar to those reported from other Middle East countries such as Saudi Arabia and Lebanon, where genotype 4 is the most prevalent [8,9]. A recently published article in Al-Najaf governorate from hemodialysis units in Al-Hakeem Hospital and Al-Sadder Teaching Hospital, patients who were HCV-RNA positive, showed genotype 4 was predominant (100%) in the examined patients [10]. However, this should not be generalized to all HCV cases prevalent in Iraq. Analysis should be done with a larger population, including hemodialysis patients and blood donors, to determine the prevalent HCV genotype [11]. HCV genotype 1a is the most prevalent genotype in Jordanian patients. This genotype is also predominant in Jordanian blood donors and in haemodialysis patients of some Middle Eastern countries including Lebanon, Turkey, Cyprus and Syria. In contrast, HCV genotype 4 is the most prevalent genotype in other Middle Eastern countries including Saudi Arabia, Egypt, Yemen and Bahrain [12]. The present study agrees with study mentioned that genotype 1a (34.48%) is more likely to disseminate in the hemodialysis environment or could be more adapted to the immunosuppression of these patients [13]. Similarly, this genotype is also predominant among hemodialysis patients of other countries such as Jordan and the United States [14,15], and 4 in Middle East countries [16], Kuwait [17], and Egypt [18]. In Syria a study found that the HCV genotypes in hemodialysis patients from Syria are equally distributed between HCV genotype 1 and HCV genotype 4 [19]. Some studies have described

super-infection in intravenous drug users and hemodialysis patients were uncommon event [20].

5. Conclusions:

It was concluded that there was no difference of HCV load in acute and chronic infections and genotype 4 as the most frequent HCV genotype in Kirkuk city.

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